

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (previously presented) A polypeptide which is a synthase or transferase obtainable from a bacterium of the family *Mycobacteriaceae*, such as of the genus

Propionibacterium which:

- (a) acts as an amide synthase or a phospho-, nucleotidyl- or aryl transferase; or
- (b) has an activity within EC 6.3.1-, EC 2.7.7-, EC 2.7.8- or EC 2.5.1.17; and/or
- (c) is obtainable from a microorganism of the Sub order *Propionibacterineae* or *Propionibacteria freudenreichii*.

Claim 2 (canceled)

3. (previously presented) The polypeptide according to claim 1 comprising:

- (i) the amino acid sequence of SEQ ID No. 2, 4, 6 or 8; or
- (ii) a variant of (i) which is a synthase or transferase; or
- (iii) a fragment of (i) or (ii) which is a synthase or transferase.

4. (previously presented) The polypeptide according to claim 3 wherein the variant in (ii) has at least 70%, 75%, 80% or 85% identity to the amino acid sequence of SEQ ID No. 2, 4, 6 or 8 and/or the fragment of (iii) is at least 150 amino acids in length.

5. (previously presented) The polypeptide according to claim 1 which is obtainable from a Gram positive bacterium and/or is a cobyrinic acid -a,c-diamide synthase, a cobinamide kinase, a cobinamide phosphate guanyltrtransferase, a cobalamin (5'-phosphate) synthase or an adenosyl transferase.

6. (currently amended) An isolated[[The]] polynucleotide comprising:

- (a) the nucleic acid sequence of SEQ ID NO:5 ~~No. 1, 3, 5 or 7~~ ~~or a sequence encoding a polypeptide according to claim 1;~~

- (b) a sequence encoding a polypeptide which is a transferase obtainable from a bacterium of the family *Mycobacteriaceae*, which acts as an aryl transferase or has an activity within EC 2.7.8.-, and
- (1) has an amino acid sequence of SEQ ID NO:6; or
- (2) is a variant of (1) having at least 70%, 75%, 80%, 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:6;
- or
- (3) is a fragment of (1) or (2), which is at least 150 amino acids in length;
- (((b))c) a sequence which is complementary to, or which hybridises to, a sequence as defined in (a) or (b);
- (((c))d) a fragment of a sequence in (a), [[or]] (b), or (c);
- (((d))e) a sequence having at least 60% identity to a sequence as defined in (a), (b), [[or]] (c), or (d); or
- (((e))f) a sequence that is degenerate as a result of the genetic code to any one of the sequences as defined in (a) to (((d))e).

7. (currently amended) The polynucleotide according to claim [[7]]6 wherein in (b) the hybridisation is under stringent conditions, the fragment in (((c))d) is at least 20 bases nucleotides in length or up to 100, 150, 200 or 300 nucleotides in length or 5 or 10 nucleotides short of the coding sequence of SEQ ID NO:5 and/or the identity in (((d))e) is at least 70% or 80%, at least 90% or 95%.

8. (currently amended) The polynucleotide according to claim 6 which comprises:

- (a) a sequence that encodes a polypeptide having synthase or transferase activity, which is:
- (1) the coding sequence of SEQ ID NO:5 ~~No. 1, 3, 5 or 7~~;
- (2) a sequence which hybridises selectively to the complement of sequence defined in (1); or
- (3) a sequence that is degenerate as a result of the genetic code with respect to a sequence defined in (1) or (2); or

(b) a sequence complementary to a polynucleotide defined in (a).

9. (previously presented) The polynucleotide according to claim 6 which is a DNA sequence.

10. (previously presented) A vector comprising one or more polynucleotide sequence(s) according to claim 6.

11. (previously presented) The vector according to claim 10 which is an expression vector.

12. (previously presented) A host cell which comprises at least one polynucleotide according to claim 6, or has multiple copies of one or more of the polynucleotide(s).

13. (previously presented) A host cell which comprises, as a heterologous sequence, a polynucleotide according to claim 6.

14. (previously presented) A host cell, optionally prokaryotic, transformed with the polynucleotide according to claim 6 or a vector comprising the polynucleotide.

15. (previously presented) A process of producing or synthesizing a polypeptide or vitamin B₁₂ or a precursor thereof, comprising culturing a host cell as defined in claim 12 under conditions that provide for expression of the polypeptide or synthesis of vitamin B₁₂ or the precursor.

16. (previously presented) A composition comprising a polypeptide according to claim 1.

17. (previously presented) A process for the preparation of an amine, comprising contacting a substrate with an amide synthase from *Propionibacteria*, or a polypeptide comprising SEQ ID No. 2, or a variant or fragment thereof as defined in claim 3.

18. (previously presented) The process according to claim 17 wherein:

- (a) the process is conducted in the presence of glutamine which is optionally converted to glutamate;
- (b) a carboxyl group is amidated to form a carboxamide group;
- (c) the substrate is cobyrinic acid or cobyrinic acid c-diamide (Formula I or IA) and/or the product of the process is cobyrinic acid c-diamide or cobyrinic acid a,c-diamide (Formula IA or IB, respectively); and/or
- (d) the process comprises amidating a substrate.

19. (currently amended) A process for the preparation of a phosphate-containing compound, the process comprising contacting a substrate with a phosphotransferase from *Propionibacterium*, a polypeptide comprising SEQ ID ~~NO:4~~NO:4 or a variant or fragment thereof as defined in claim 3.

20. (previously presented) The process according to claim 19 wherein:

- (a) the process is conducted in the presence of a nucleoside triphosphate, such as ATP;
- (b) the substrate comprises adenosine;
- (c) the process comprises phosphorylation, optionally of a hydroxyl group; and
- (d) the substrate comprises adenosyl cobinamide (Formula II) and/or the product of the reaction is adenosyl cobinamide phosphate (Formula IIA).

21. (previously presented) A process for the preparation of a nucleotidyl-containing compound, the process comprising contacting a substrate with a nucleotidyl transferase from *Propionibacterium*, a polypeptide comprising SEQ ID No. 4 or a variant or fragment thereof as defined in claim 3.

22. (previously presented) The process according to claim 21 wherein:

- (a) the process comprises guanidylating substrate;

- (b) the process comprises nucleotidylating a phosphate group;
- (c) the process is conducted in the presence of a nucleosyl triphosphate, such as GTP; and/or
- (d) the substrate comprises adenosyl cobinamide phosphate (Formula IIA) and/or the product of the reaction is adenosyl-GDP-cobamide (Formula IIB).

23. (previously presented) A process for the preparation of an aryl-containing compound, the process comprising contacting a substrate with an aryl transferase from *Propionibacterium*, a polypeptide comprising SEQ ID No. 6 or a variant or fragment thereof, as defined in claim 3.

24. (previously presented) The process according to claim 23 wherein:

- (a) the aryl moiety comprises an aromatic ring system of one or two rings, optionally substituted with 1 to 4 C₁₋₈ alkyl groups, and with 0, 1 or 2 heteroatoms, optionally benzimidazole;
- (b) the product of the reaction has the aryl group bound to a transition metal, such as cobalt, and to a carbon atom, optionally also to a ribose group;
- (c) the process is conducted in the presence of a ribazole; and/or
- (d) the substrate comprises adenosyl-GDP-cobamide (Formula IIB) and/or the product comprises adenosyl-5,6-dimethyl benzimidazolyl cobamide (vitamin B₁₂, Formula IIC).

25. (previously presented) A process for the preparation of an adenosine-containing compound, the process comprising contacting a substrate with an adenosyl transferase from *Propionibacterium*, or a polypeptide comprising SEQ ID No. 8 or a variant or fragment thereof as defined in claim 3.

26. (previously presented) The process according to claim 25 wherein:

- (a) the process comprises adenosylating a substrate, or the transfer of adenosine;

- (b) the process involves the bonding of adenosine to a metal atom, optionally a transition series metal;
- (c) is conducted in the presence of a nucleosyl (tri) phosphate; and/or
- (d) the substrate comprises cobyrinic acid a,c-diamide (Formula IB) and/or the product comprises adenosyl cobyrinic acid -a,c-diamide (Formula IC).

27. (previously presented) A process for producing vitamin B₁₂ or a precursor thereof, the process comprising culturing or fermenting a host cell according to claim 12 under conditions such that vitamin B₁₂ or the precursor is produced or synthesised.

Claim 28 (canceled)

29. (currently amended) The[[A]] vector according to claim 10 comprising:

- (a) a polynucleotide encoding a polypeptide wherein said polypeptide acts as a (phospho)transferase or a (nucleotidyl)transferase or has an activity within EC 2.7.1.- or EC 2.7.7.- or is the amino acid sequence of SEQ ID NO:4 or a variant of said SEQ ID NO:4 or a fragment of said SEQ ID NO:4 or has at least 70%, 75%, 80% or 85%, 90%, or 95% identity to the amino acid sequence of SEQ ID NO:4, or the nucleic acid sequence SEQ ID NO:3; and
- (b) a polynucleotide encoding a polypeptide wherein said polypeptide acts as an (aryl)transferase or has an activity within EC 2.7.8.- or it is the amino acid sequence of SEQ ID NO:6 or a variant of said SEQ ID NO:6 or a fragment of said SEQ ID NO:6 or has at least 70%, 75%, 80% or 85%, 90% or 95% identity to the amino acid sequence of SEQ ID NO:6, or the nucleic acid sequence SEQ ID NO:5.

30. (currently amended) The[[A]] vector according to claim 10 further comprising a nucleic acid sequence encoding the CobA protein.

31. (currently amended) The[[A]] vector according to claim 30 wherein the nucleic acid sequence encoding the CobA protein is from *P. freudenreichii*.
32. (previously presented) The polynucleotide according to claim 7 wherein the fragment is at least 510 bases for a fragment of SEQ ID No. 7 and/or the identity is at least 85% for SEQ ID No. 7.
33. (currently amended) The vector ~~[[of]]~~according to claim 11 wherein the polynucleotide is a DNA sequence operably linked to a regulatory sequence.
34. (previously presented) A process for the preparation of an amine, comprising contacting a substrate with a host cell as defined in claim 12.
35. (previously presented) A process for the preparation of a phosphate-containing compound, comprising contacting a substrate with a host cell as defined in claim 12.
36. (previously presented) A process for the preparation of a nucleotidyl-containing compound, comprising contacting a substrate with a host cell as defined in claim 12.
37. (previously presented) A process for the preparation of an aryl-containing compound, comprising contacting a substrate with a host cell as defined in claim 12.
38. (previously presented) A process for the preparation of an adenosine-containing compound, comprising contacting a substrate with a host cell as defined in claim 12.
39. (previously presented) The process of claim 26 wherein the nucleosyl (tri)phosphate is ATP and the transition series metal is cobalt.
40. (currently amended) The[[A]] vector according to claim 10 comprising:

- (a) a polynucleotide encoding a polypeptide wherein said polypeptide acts as a (phospho)transferase or a (nucleotidyl)transferase or has an activity within EC 2.7.1.- or EC 2.7.7.- or is the amino acid sequence of SEQ ID NO:4 or a variant of said SEQ ID NO:4 or a fragment of said SEQ ID NO:4 or has at least 70%, 75%, 80% or 85%, 90% or 95% identity to the amino acid sequence of SEQ ID NO:4, or the nucleic acid sequence SEQ ID NO:3; and
- (b) a polynucleotide encoding a polypeptide wherein said polypeptide acts as an (aryl)transferase or has an activity within EC 2.7.8.- or it is the amino acid sequence of SEQ ID NO:6 or a variant of said SEQ ID NO:6 or a fragment of said SEQ ID NO:6 or has at least 70%, 75%, 80% or 85%, 90% or 95% identity to the amino acid sequence of SEQ ID NO:6, or the nucleic acid sequence SEQ ID NO:5 and further comprising a nucleic acid sequence encoding the CobA protein.

41. (currently amended) The[[A]] vector according to claim 40 wherein the nucleic acid sequence encoding the CobA protein is from *P. freudenreichii*.

42. (new) The polynucleotide according to claim 6 which further comprises:

- (a) the nucleic acid sequence of SEQ ID NO:3;
- (b) a sequence encoding a polypeptide which is a transferase obtainable from a bacterium of the family *Mycobacteriaceae*, which acts as a nucleotidyl or phospho transferase or has an activity within EC 2.7.1.-, EC 2.7.7.-, and
 - (1) has an amino acid sequence of SEQ ID NO:4; or
 - (2) is a variant of (1) having at least 70%, 75%, 80%, 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:4; or
 - (3) is a fragment of (1) or (2), preferably which is at least 150 amino acids in length;
- (c) a sequence which is complementary to, or which hybridizes to, a sequence as defined in (a) or (b);
- (d) a fragment of a sequence in (a), (b), or (c);

- (e) a sequence having at least 60% identity to a sequence as defined in (a), (b), (c), or (d); or
- (f) a sequence that is degenerate as a result of the genetic code to any one of the sequences as defined in (a) to (e).

43. (new) A vector comprising one or more polynucleotide sequence(s) according to claim 42 and wherein the vector optionally further comprises a nucleic acid sequence encoding a CobA protein, wherein the CobA protein is preferably derived from *Propionibacterium freudenreichii*.

44. (new) A host cell which comprises at least one polynucleotide according to claim 42 or has multiple copies of one or more of the polynucleotide(s).

45. (new) A host cell, optionally prokaryotic, transformed with a vector according to claim 43.

46. (new) A process of producing or synthesizing a polypeptide or vitamin B₁₂ or a precursor thereof, comprising culturing a host cell as defined in claim 45 under conditions that provide for expression of the polypeptide or synthesis of vitamin B₁₂ or a precursor thereof.